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EDGEWOOD ARSENAL, ABERDEEN PROVING GROUND, MARYLAND

DECEMBER 1976

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EDGEWOOD ARSENAL TECHNICAL REPORT

EB-TR-76097

IN VITRO INHIBITION OF MICROSOMAL CALCIUM ATPASE FROM EGGSHELL GLAND OF MALLARD DUCK

by

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December 1976

DEPARTMENT OF THE ARMY
Headquarters, Edgewood Arsenal
Aberdeen Proving Ground, Maryland 21010

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The results of this study have demonstrated the inhibition of Ca ATPase by DDT in an *in vitro* microsomal test system. Furthermore, this inhibition has been established to be competitive in nature. Our results are similar to those of other investigators who showed Ca ATPase inhibition by DDE in shell gland homogenate. Previous studies have shown DDT-induced eggshell thinning to be due to decreased calcium content of eggshells. Since calcium ATPase has been shown to be associated with calcium transport in eggshell gland, the inhibition of this enzyme *in vitro* offers a possible explanation for DDT-induced eggshell thinning.

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PREFACE

The work described in this report was authorized under Project/Task No. EPA. This work was started in February 1976 and completed in March 1976.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animals Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

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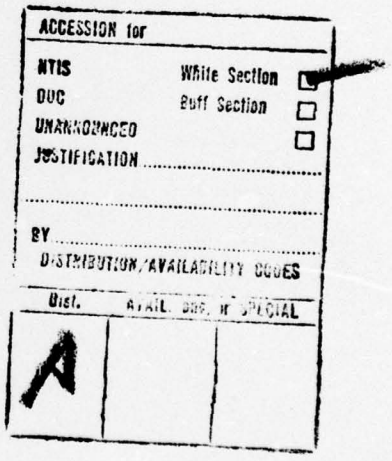

IN VITRO INHIBITION OF MICROSOMAL CALCIUM ATPASE FROM EGGSHELL GLAND OF MALLARD DUCK

I. INTRODUCTION

In a previous paper we reported the effects of 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane (DDT) on morphology of eggshell gland of the mallard duck (*Anas platyrhynchos*) (Kolaja and Hinton 1976).[1] In that study, edema of the eggshell gland mucosa accompanied eggshell thinning. We postulated that the edema could have arisen as the result of an ionic imbalance. Calcium adenosine triphosphatase (ATPase) is the enzyme which is thought to be responsible for the transport of one ion, calcium, across epithelium of the eggshell gland (Pike and Alvarado 1975).[2] Both *in vivo* and *in vitro* inhibition of Ca ATPase by the DDT metabolite 2,2-bis-(chlorophenyl)-1,1-dichloroethylene (DDE) has been demonstrated in homogenate of freeze-dried mucosal preparation from shell gland of Pekin ducks (Miller, *et al.*, 1976).[3] The primary purpose of this study was to determine the inhibitory effect of DDT on Ca ATPase isolated from microsomal preparations of duck eggshell glands and to characterize the type of inhibition by Lineweaver/Burke plots of Michaelis Menten analyses.

II. MATERIALS AND METHODS

Mature mallard hens, in egg production, were killed by cervical dislocation and the eggshell glands were rapidly excised and placed in ice-cold 0.25 M sucrose. All subsequent procedures were done in an ice bath or at 4°C. The mucosa of the eggshell gland was removed by scraping with a clean razor blade and 1 gm was weighed and diluted up to 10 ml in 0.25 M sucrose. The sucrose-shell gland mixture was homogenized at 500 rpm in a Potter-Elvehjem tissue homogenizer. The homogenate was sedimented for 15 minutes at 18,000 X g max in a Beckman L-2 ultracentrifuge. The above supernatant was recentrifuged at 98,000 X g max for 1 hr. The resulting pellet was resuspended to 3 ml in 0.25 M sucrose, and stored at -60°C until the time of assay. The Ca ATPase activity was determined by the method of Rorive and Kleinzeller (1974).[4] Twenty microliters of DDT (Pfaltz and Bauer, Flushing, New York), dissolved in acetone were added to the reaction mixture and brought to a final concentration of 10^{-4}, 0.5×10^{-4}, and 10^{-5} gm/ml in a final volume of 2 ml of reaction mixture. Control preparations received an equal volume of acetone without DDT. Preliminary experiments revealed no enzyme inhibition with the above volume of acetone alone. Tris salt of adenosine triphosphate (ATP) (Sigma Chemicals) was used in concentrations of 2.5 mM, 1.25 mM and 0.625 mM. The phosphate released at the end of 30 minutes was determined by the method of Stanton (1968).[5]

III. RESULTS

The inhibitory effect of DDT on Ca ATPase is shown in figure 1. When 1/velocity was plotted against inhibitor concentration, the inhibitor binding constant (I) was approximately 8.8×10^{-4} gm/ml. The competitive nature of the inhibition of Ca ATPase by DDT is shown when the reciprocal of velocity is plotted versus the reciprocal of substrate concentration (figure 2). This plot shows that the inhibition by DDT was overcome by addition of more substrate (ATP).

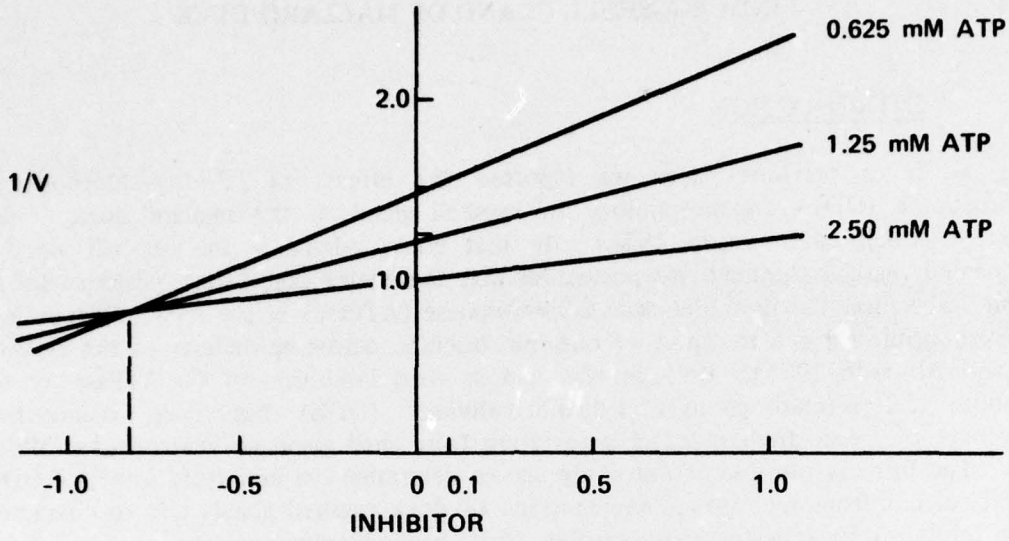

Figure 1. *In Vitro* Ca ATPase Inhibition by DDT

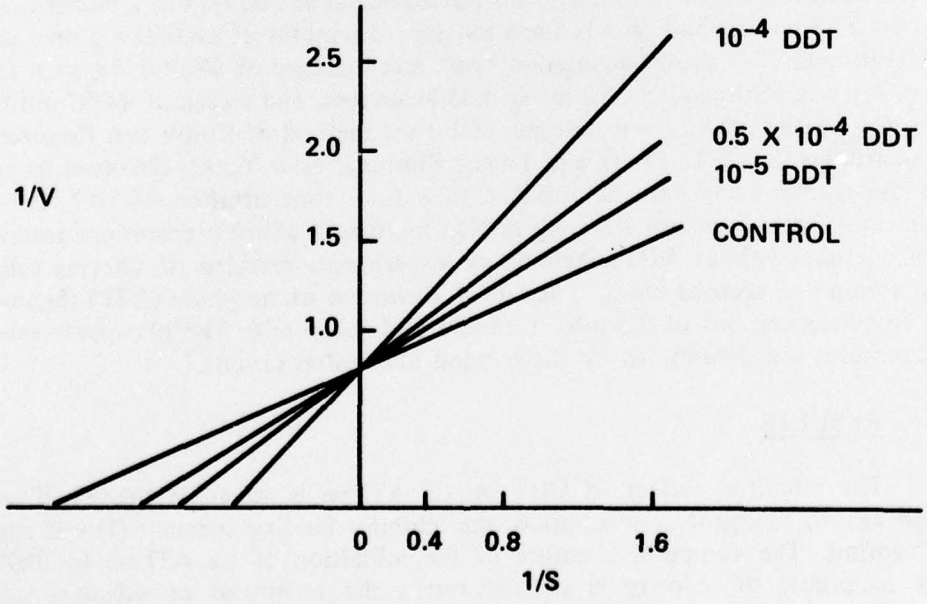

Figure 2. Lineweaver-Burke Plot of Ca ATPase Inhibition by DDT

IV. DISCUSSION

The results of this study have demonstrated the inhibition of Ca ATPase by DDT in an *in vitro* microsomal test system. Furthermore, this inhibition has been established to be competitive in nature. Our results are similar to those of Miller, *et al.*, (1976)[3] who showed Ca ATPase inhibition by DDE in shell gland homogenate. Previous studies have shown DDT-induced eggshell thinning to be due to decreased calcium content of eggshells (Bitman, *et al.*, 1969).[6] Since calcium ATPase has been shown to be associated with calcium transport in the eggshell gland, the inhibition of this enzyme *in vitro* offers a possible explanation for DDT-induced eggshell thinning.

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